EVALUATION OF ANTIDIABETIC, HYPOLIPIDEMIC AND ANTIOXIDANT ACTIVITY OF SEAWEED *HALIMEDA MACROLOBA*

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Submitted to

SRI BALAJI VIDYAPEETH

Submitted by

Ms. Kavya R

UIN: [2105003001]

Under the Guidance of

Dr.Manimekalai K

Professor and Head, Department of Pharmacology

MGMCRI, Pillaiyarkuppam, Puducherry - 607 402.

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ABSTRACT

Background:

The seaweed *Halimeda macroloba* is a great source of biologically active secondary metabolites. In vitro studies have demonstrated multiple therapeutic benefits. However, no studies have examined its in vivo activity. Thus, the current investigation is being conducted to investigate the antioxidant, hypolipidemic, and antidiabetic effects of the hydroalcoholic extract of *H. macroloba* (HME) in Wistar rats.

Aim and Objectives:

To evaluate the antidiabetic, hypolipidemic and antioxidant activity of seaweed *H.macroloba* in Wistar albino rats

Materials and Methods:

HME was prepared using 70% alcohol as a solvent using maceration and Soxhlet extraction for 72 and 48 hours and proceeded with phytochemical analysis, toxicity study and then evaluated the antidiabetic, hypolipidemic and antioxidant activity. Nicotinamide (i.p.,110mg/kg) and Streptozotocin (i.p.,45mg/kg) were administered to induce diabetes. Rats were randomly divided into seven groups and treated with two doses of HME and conventional medications. Weekly monitoring of body weight and fasting blood glucose with monthly lipid profiles, antioxidant parameters, HbA1c, insulin, and IR were measured on the 90^{th} day. Histopathological analysis was performed on the renal, liver and pancreatic tissues. After this, molecular docking and dynamic simulation studies were performed to confirm the binding affinity.

Results:

Pharmacologically active phytoconstituents were identified by qualitative and quantitative analysis. There were no significant abnormalities or fatalities from the acute and chronic toxicity study of HME. Histopathological investigations revealed that HME had no adverse effects on the vital organs. In antidiabetic, hypolipidemic and antioxidant studies showed significant improvements in body weight, Fasting Blood Glucose, insulin levels and a reduction in HbA1c, and insulin resistance following treatment with HME. Similarly, HME substantially raised HDL levels while lowering LDL, TC, and TG. Comparing the HME groups to the untreated diabetic rats, there was a significant rise in the antioxidant levels of GSH, CAT and SOD. However, compared to diabetic rats, HME groups had lower levels of MDA. Comparably, HME reversed the histological

alterations and restored the liver, kidney, and pancreas functions. Molecular docking and dynamic simulation results also showed significant binding affinity.

Conclusion:

The overall results show administering HME improved BW, HbA1c, insulin levels and glycaemic management. Additionally, the lipid profiles and antioxidant activities have been considerably enhanced. and hence confirmed that *H.macroloba* has significant antidiabetic, hypolipidemic and antioxidant activity.

Keywords: Antidiabetic, Antioxidant, *Halimeda macroloba*, Hypolipidemic, Molecular docking, Toxicity study.

1. INTRODUCTION

Diabetes is a chronic metabolic condition accompanied by elevated blood glucose levels, according to the World Health Organization (WHO).[1] Moreover, diabetes mellitus (DM), which further manifests as hyperglycemia, can harm several systems and result in organ failure including the heart, blood vessels, eyes, kidneys, and nerves, if left untreated.[2] The most common sort of diabetes is type 2 diabetes which primarily affects adults and is brought on by insufficient or resistant insulin production in the body. Inactivity, being overweight, and leading an unhealthy lifestyle are the main causes of type 2 diabetes and it was once primarily associated with adults, but it increasingly affects the young. Type 2 diabetes has been considerably more prevalent over the last three decades in all nations, regardless of wealth. Approximately 422 million individuals globally suffer from diabetes, and the disease is directly responsible for 1.5 million fatalities annually. Over the past few decades, there has been a steady rise in both the number of cases and the incidence of diabetes.[1] As per the 2021 release by the IDF Diabetes Organization, about 537 million adults worldwide have diabetes. This figure is anticipated to increase to 643 million by 2030 and 783 million by 2045.

As far, as a precise drug or total cure for diabetes has not been found. Diabetes can be managed with blood sugar monitoring, a healthy lifestyle change, and physical activity, yoga, cycling, jogging, or any other activity that the patient chooses. The majority of type 1 diabetes treatment consists of injecting exogenous insulin and using oral drugs to maintain a healthy lifestyle.[3] Typically, metformin is used for type 2 diabetes.

Numerous academic studies have demonstrated the connection between the pathophysiology of diabetes mellitus and the production of free radicals. Multiple studies revealed that decreased levels of endogenous antioxidants and increased free radical production led to the demise of high lipid peroxidation, pancreatic β-cells, oxidative stress, and cellular organelle impairment. These events ultimately cause secondary complications such as neuropathies, nephropathies, diabetes-related retinopathies, and cardiovascular diseases.[4] Since diabetes is thought of as a chronic metabolic condition, many conventional antidiabetic medications do not include a single dose because most of the drugs require periodic injections. However, a number of these conventional medicinal products are ineffective and have significant adverse consequences.[5] Due to these drawbacks, research into management techniques utilizing medicinal plants, which are reportedly less expensive antidiabetic medicines with less documented adverse effects, has intensified.[6]

Several studies on seaweeds have revealed the presence of phytochemical components that are responsible for regulating and overcoming diabetes. Consuming edible seaweeds regularly and incorporating them into your diet has been associated with a lower risk of diabetes. Many polyphenolic chemicals widely known for their ability to prevent diabetes have been identified from seaweed.[7]

Among such seaweed, *Halimeda macroloba* (*H.macroloba*) is a green macroalgae from the family of Chlorophyceae. The algal body (thallus) is composed of calcified green segments which is comparatively new species.

Halimeda macroloba

Taxonomical Classification:

Botanical Name: *Halimeda macroloba*

Kingdom : *Plantae*

Subkingdom : *Viridiplantae*

Class : *Chlorophyta*

Order : *Bryospidales*

Family : *Halimedaeceae*

Genus : *Halimeda*

Species : *Halimeda macroloba Decaisne*

H.macroloba has *in vitro* α – glucosidase and cytotoxic properties,[8] antioxidant activity [9] and antibacterial activity.[10] It has various therapeutic qualities that have been investigated; however, adequate investigations have not been undertaken to utilize its pharmacological activities and potential mechanism of action. Based on the *invitro* information that was already available and knowledge from earlier facts, our study aimed to scientifically validate the antidiabetic, hypolipidemic, and antioxidant activity of seaweed *Halimeda macroloba* in Wistar albino rats.

2. AIMS AND OBJECTIVES

To evaluate the antidiabetic, hypolipidemic and anti-oxidant effect of *Halimeda macroloba* using streptozotocin-Nicotinamide induced type 2 diabetes mellitus in Wistar albino rats with the following objectives

1. To evaluate acute and chronic toxicity of hydroalcoholic extract of *Halimeda macroloba* (HME)
2. To evaluate the anti-diabetic effect of hydroalcoholic extract of *Halimeda macroloba* and compare it with metformin and acarbose.
3. To evaluate the hypolipidemic effect of hydroalcoholic extract of *Halimeda macroloba* and compare it with atorvastatin.
4. To evaluate the anti-oxidant property of the hydroalcoholic extract of *Halimeda macroloba.*
5. To evaluate the binding affinity of ligands of *Halimeda macroloba* and standard drugs against the proposed protein targets.

3. HYPOTHESIS/RESEARCH QUESTION

Research Question: Does the hydroalcoholic extract of *Halimeda macroloba* have significant antidiabetic, hypolipidemic and antioxidant activity?

Null hypothesis: The hydroalcoholic extract of *Halimeda macroloba* does not possess significant antidiabetic, hypolipidemic and antioxidant activity.

Alternative hypothesis: The hydroalcoholic extract of *Halimeda macroloba* possesses significant antidiabetic, hypolipidemic and antioxidant activity.

4. REVIEW OF LITERATURE

One of the main causes of type-2 diabetes progression is beta-cell dysfunction. A serious public health issue that significantly affects both the lives of individuals and the expenses of medical care. Rapid urbanisation and economic growth are rapidly causing the disease to become prevalent despite tremendous clinical care efforts, public health initiatives, and research.[11,12] Growing calls for the search for more effective treatment alternatives with few or no side effects are a reflection of the inefficiency provided by conventional antidiabetics and the expanding worldwide incidence of this illness.[13] Marine natural materials have drawn increasing attention in recent years as a possible source of novel medications to treat diabetes and its related problems.[14] Because of their distinct chemical profile and therapeutic qualities, seaweeds stand out among the wide variety of marine life.[15] Numerous bioactive substances produced by seaweeds, such as peptides, polysaccharides, and polyphenols, have shown antidiabetic effects in preclinical research.[16] It has been demonstrated that seaweed-derived polysaccharides promote insulin sensitivity, boost glucose absorption, and lower blood glucose levels.[17] Further, the polyphenols in seaweeds diminish postprandial blood glucose levels by blocking the action of α-glucosidase, an enzyme that converts complex carbs to glucose.[18]

Palanisawamy et al., 2020 have reported that Free radical scavenging tests revealed the antioxidant activity of *Ulva fasciata Delile* due to the presence of sesquiterpenoids. Flavonoids are rich in *Ulva lactuca* and have antioxidant properties. Extraction of *Ulva reticulata* using hot water reduced hepatic oxidative stress.

Polysaccharides isolated from *U.lactuca* could significantly decrease blood glucose by their potential inhibitory effect on key enzymes closely related to starch digestion and absorption in both plasma and small intestine. In another study, the ethanol extract of *Ulva rigida* was reported to decrease the blood glucose concentration and occurrence of micronuclei in diabetic rats.

Gotama et al., 2018 evaluated *Sargassum hystrix* extracts for their antidiabetic activity in Streptozotocin (STZ) – induced diabetic rat model. It was reported that the pre-prandial and postprandial glucose levels were reduced, and the levels of triglycerides and cholesterol were reduced in the group treated with 300 mg/kg extract than the positive control group. However, it was reported that the levels of high-density lipoprotein cholesterol and low-density lipoprotein cholesterol in the seaweed extract-treated group showed the best capability to reduce pre-prandial and postprandial glucose and also prevent necrosis in diabetic rats.

Sapaore et al., 2006 *Chlorella* acts as an active immune stimulation for free radical and blood cholesterol reduction. The effect of this compound in curing gastric ulcers, sores and constipation has been studied. It has also been demonstrated to prevent the occurrence of diseases such as atherosclerosis and hypercholesterolemia and proved to have some antitumor activity.

Nazarudin et al., 2020 have conducted a study to evaluate the α-Glucosidase Inhibitory and Cytotoxic Properties of Marine Algae *Ulva intestinalis, Halimeda macroloba*, and *Sargassum ilicifolium.* According to their findings, *U. intestinalis* had the highest quantities of total carotenoids (162.00 μg g−1 DW), chlorophyll a (313.09 ± 2.53 μg g−1 DW), and chlorophyll b (292.52 ± 8.84 μg g−1 DW). Compared to other seaweed species, *S. ilicifolium* showed the lowest efficacy in the α-glucosidase inhibitory activity, with an IC50 value of 38.491 ppm. However, compared to other cell lines tested, *H. macroloba* extract was the most cytotoxic to MCF-7 and HT 29 cells, with IC50 values of 37.25 ± 0.58 and 21.32 ± 0.25 μg/mL, respectively. With an IC50 of 48.80 ± 0.11 μg/mL, *H. macroloba* extract was also shown to be less harmful to normal cells (3T3).

The extract from *U. intestinalis* showed the greatest cytotoxicity to Hep G2 cells, with an IC50 of With an IC50 of 25.23 ± 0.11 μg/mL, *S. ilicifolium* was less hazardous to MDA-MB231 cells than 23.21 ± 0.11 μg/mL. Following that, 27 metabolites in *U. intestinalis*, 22 in H. macroloba, and 24 in *S. ilicifolium* were identified using GC-MS analysis of the methanolic extracts of these seaweed samples. When combined, the study's findings showed that every seaweed species under evaluation is a suitable prospect for production as a functional food source for human consumption and should be encouraged.

Most seaweeds have already been explored for their biopharmaceutical properties and play a significant part in controlling chronic diseases. The chemicals found in seaweed have the potential to be used in human health applications. The capacity to modify gut health and risk factors for obesity and diabetes, as well as antiviral, anticancer and anticoagulant characteristics, are among the purported advantages. Since *Halimeda macroloba* (marine green algae) is comparatively a new species which is not been explored for its bioactive and pharmacological properties. So, the present study was the first to be carried out to standardise and scientifically evaluate the antidiabetic, hypolipidemic and antioxidant activity of the hydroalcoholic extract of *Halimeda macroloba* through in vivo studies. Scientific information generated from this research work may add strong evidence about the potential use of the extract in the management of diabetes, hyperlipidaemia and oxidative stress.

5. METHODOLOGY

PHASE - I

PLANT MATERIAL:

Collection and identification of the plant material

The seaweed *H. macroloba* was collected in November 2022 from the seashore of Vedali, Mandapam, Rameswaram, India. Seaweeds were authenticated with a marine biologist's assistance and have been authenticated from CAS Marine Biology, Parangipettai, Tamilnadu, India and provided with a Voucher herbarium no: CAS MBMBTL02.

Preparation of hydroalcoholic extract

The harvested seaweed was rinsed with water from the faucet to remove dirt and shade-dried at room temperature (24 ± 2 °C) for 6–7 weeks. The dried seaweed was utilised for further examination after being thoroughly dried and processed into a coarse powder using a mixer grinder and sieve. It was then kept in airtight containers. The Extracts were prepared using 70 % alcohol as solvent by the method of maceration for 72 h and Soxhlet extraction for 48 hours.[12] With the use of Whatman No. 42 filter paper, the crude extract solution was purified. After this, the yield was Concentrated by lyophilization to obtain an *H. macroloba* hydroalcoholic extract (HME) and it was kept in a sealed container at 20 °C to undergo additional examination. The extract was mixed with distilled water and given to rats orally.

Phytochemical analysis

HME was screened qualitatively and quantitatively for the existence of several phytochemical classes, including **tannins, saponins, terpenoids, steroids, flavonoids, alkaloids, phenols, Coumarins, Quinones, and Glycosides** using standard procedure.[22,23,24]

Evaluation of Acute Toxicity Study of Seaweed *H.macroloba*

Ethical Clearance and IAEC Approval:

Following clearance by the institutional animal ethics committee (IAEC) of MGMCRI, SBV University, Puducherry (CPCSEA regd. No.: 686/PO/Re/S/02/CPCSEA, 21-08-2002) and approved proposition no.: 02/IAEC/MG/10/2022-III, the acute toxicity investigation was being carried out.

Experimental animals:

A total of 12 female, non-pregnant Wistar albino rats, weighing 170-220 gm were utilized, as the female rats are more sensitive than the males. Albino rats were procured from the CPCSEA-approved animal breeding centre and the rats were housed in the central animal house of MGMCRI.

Animals were quarantined for a minimum of one week in different rooms before testing. Under conventional laboratory conditions, animals were housed in hygienic polypropylene cages with stainless steel top grills (temperature, 25 ± 2 °C; relative humidity, 65%; light/dark cycle, 12/12 hours). To make the bedding, fresh paddy husk which was dedusted and autoclaved is utilised. Reverse osmosis (Rios, USA) water was available to the animals at all times in hygienic polypropylene bottles with stainless steel sipper tubes. The animals were fed regular rat pellet feed (Amrut Laboratory Animal Feed, Pune). Daily cleanings were performed on the water bottles and cage bedding and the study was carried out in compliance with the OECD Chemical Testing Guidelines, 423.

Evaluation of Acute toxicity study of seaweed *H.macroloba* in Wistar albino rats

By recommendations provided by the Organisation for Economic Co-operation and Development (OECD) 423, acute toxicity experiments were carried out on February 2023 in Wistar albino rats.[25] A total of 12 female non-pregnant Wistar albino rats were randomized into four groups of three rats each (n=3). The first group was the normal control group and received distilled water, the second, third and fourth were treatment groups and received 50, 300 and 2000 mg/kg doses of *H. macroloba* extract (HME). Before the commencement of the experiment, all of the rats were weighed, identified by markings, and granted unrestricted access to water before the experiment started. Following the dose, the rats fasted for an additional four hours. Observations were made in real time for every rat in each group for the first four hours following the drug treatment, and for an additional twenty-four hours afterwards, to look for suspicious changes or mortality. After that, they were monitored twice a day for a total of fourteen days to look for any hazardous consequences, such as changes in the colour of the skin, eyes, or hair, changes in how much food and liquids they consumed, tremors, convulsions, salivation, diarrhoea, lethargy, breathing, abnormal behaviour, motor activity, sleep, or coma. The goal of the acute toxicity test was to determine the test extract's short-term toxicity level, which is useful in determining dosages for subsequent research.

On the 14th day, the blood samples were collected by cardiac puncture, then haematological and biochemical analysis was done for CBC, liver profile (SGPT, SGOT, Alkaline Phosphate), and renal profile (urea, creatinine). The same day all the treatment and control group rats were sacrificed by giving an Isoflurane and a complete necropsy was done. The liver, kidney, and pancreas were dissected and preserved by using 10% formalin for routine histopathological examination.

STATISTICAL ANALYSIS

Biochemical findings were tabulated and statistically analysed. The analysis was done by using STATA software version 17 and represented as standard deviation. The findings were calculated and expressed as Mean ± Standard deviation. The data obtained in the investigations were subjected to a one-way analysis of variance (ANOVA) to determine the significant difference. The Bonferroni's t-test was used to evaluate the inter-group significance. Significant differences were defined as 5% level ($p \leq 0.05$) of significance.

Evaluation of Chronic Toxicity Study of Seaweed *H.macroloba* in wistar albino rats

Ethical Clearance

The study was commenced after approval from the institutional animal ethics committee (IAEC) of MGMCRI, SBV University, Puducherry (CPCSEA regd. No.: 686/GO/Re/S/02/CPCSEA(2022-2027). And approved proposition no.: 09/IAEC/MG/04/2023 – I.

Experimental Animals:

Animals consisting of adult male (n=30) and adult female (n=30) Wistar rats weighing between 150 to 200 g were procured from Biogen Laboratory Animal Facility, Bangalore (CCSEA-approved breeding facility) and it was housed at MGMCRI's primary animal house. Animals were acclimatized for one week before testing and animals were housed in hygienic polypropylene cages under standard laboratory settings (25 ± 2 °C, 65 % relative humidity, and 12/12 light/dark cycle). All the animals were fed with a normal pellet diet and drinking water was given *ad libitum*. The study animals were handled according to the CCSEA and OECD (test no:452) Chemical Testing Guidelines.

Chronic Toxicity Study:

The chronic toxicity study was done as per the OECD 452 guidelines.[26] Following the acute toxicity study, Wistar albino rats of either sex (n=40; 20 male and 20 female rats) were divided into four groups each having 10 animals (5 males and 5 Females) were administered hydroalcoholic extract of *H.macroloba* (HME) as follows: Group I (Control group), Group II to IV were orally administered with HME at dose of 250 mg/kg, 500mg/kg and 1000 mg/kg BW respectively. The other 2 groups of rats {n=10 (5+5)} served as satellite control and satellite groups of the chronic dose toxicity study observations of HME. The maximum (Volume) dose of HME that can be administered was fixed at 1000 mg/kg BW

After overnight fasting, the animals were weighed and the HME was administered orally through an oral feeding needle, at the dose of 250, 500 and 1000 mg/kg BW daily for 180 days. After the test drug had been administered, food was restricted for 3-4 hours. Animals were observed

individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, and daily for 180 days. Individual animal behavioural changes were observed and recorded daily for 180 days. The animals were monitored daily for any toxicity.

To access reversibility, In the satellite group, the animals were dosed with the safer highest dose (1000 mg/kg BW) from the chronic toxicity study and the control group to monitor and compare any specific observations of toxic changes in the animals during 180 days and no treatment for the following 28 days. The toxic manifestations such as signs of toxicity, mortality, and body weight changes were monitored daily.

Haematological analysis:

On the 90^{th} and 180^{th} day, the blood samples (2ml) collected in EDTA blood samples were subjected to a Complete Blood Count (CBC) using a Veterinary Haematology analyser

Biochemical analysis:

On the 90^{th} and 180^{th} day, Serum samples were subjected to the analysis of blood glucose, bilirubin, creatinine, cholesterol, albumin, globulin, SGOT, SGPT, alkaline phosphatase, albumin and globulin ratio (A/G ratio) using a biochemical autoanalyzer.

Histopathological Examination:

After blood collection the animals were sacrificed using a carbon-di-oxide chamber, and major organs like the heart, liver, kidney, pancreas, and spleen in all animals, ovaries from female animals and testis from male animals were collected to observe for any changes. After the collection, the organs were fixed in 10% neutral buffered formalin and processed by conventional techniques. Paraffin sections were stained with H&E and observed under 40x magnification for any change in morphology and cellular damage.

Statistical analysis

All the data were analyzed using the SPSS software version. 26.0. Results were statistically analysed via a one-way analysis of variance (ANOVA) and a posthoc test Bonferroni's was performed. All experimental data are expressed as the mean ± Standard Deviation (SD). Significant differences were accepted at * $p < 0.05$ at a 95% confidence interval.

PHASE - II

Evaluation of Antidiabetic, hypolipidemic and antioxidant activity of seaweed *Halimeda macroloba* in Wistar albino rats

Ethical Clearance

The study was commenced after approval from the institutional animal ethics committee (IAEC) of MGMCRI, SBV University, Puducherry (CPCSEA regd. No.: 686/GO/Re/S/02/CPCSEA(2022-2027). And approved proposition no.: 08/IAEC/MG/04/2023 – I.

Chemicals and equipment:

Streptozotocin and Nicotinamide were procured from Sigma Aldrich Chemicals, Mumbai. Metformin, Acarbose, Atorvastatin and 70% alcohol were procured from MGMCRI pharmacy. A glucometer manufactured by one touch was used for the estimation of blood glucose. All other reagents and chemicals used were of analytical grade.

Animals

Animals consisting of adult male Wistar rats (n=42), weighing between 150 to 200 g were procured from Biogen Laboratory Animal Facility, Bangalore (CCSEA-approved breeding facility) and housed at MGMCRI's primary animal house. Animals were acclimatized for at least one week before testing. Animals were housed in hygienic polypropylene cages under standard laboratory settings (25 ± 2 °C, 65 % relative humidity, and 12/12 light/dark cycle). The bedding is made from fresh paddy husk. Animals were fed with a normal pellet diet and drinking water was given *ad libitum*. The study animals were handled according to the CCSEA and OECD Chemical Testing Guidelines.

Induction of Type 2 Diabetes Mellitus

In overnight fasted rats, Nicotinamide (NA) was injected intraperitoneally at a dose of 110 mg/kg body weight (BW) 15 minutes prior to the injection of streptozotocin. Following that, 45 mg/kg BW of streptozotocin (STZ) in 0.1M cold sodium citrate buffer was given. Blood glucose levels were assessed 72 hours after the STZ-NA injection, and rats exhibiting blood glucose levels greater than 250 mg/dl were considered diabetic and utilized in the following experiment.

Experimental Design

Then the animals were randomly divided into 7 groups with 6 rats in each (a total of 42 rats) and treated with (i) normal saline, (ii) Diabetic control (iii) 100 mg/kg body weight (BW) of the hydroalcoholic extract of *H.macroloba* (HME), (iv) 200 mg/kg BW HME, (v) 100 mg/kg BW metformin, (vi) 12.5 mg/kg BW Acarbose, (vii) 2.5 mg/kg BW Atorvastatin + 100 mg/kg BW Metformin. The extract and drug were reconstituted in distilled water, and treatments were

administered daily for 90 days through the oral route with the aid of an oral gavage needle. The extract doses were selected based on the literature and our pilot studies ($1/10^{th}$ and $1/20^{th}$ of acute toxicity study of seaweed *H.macroloba* were used). The rat's Body weight was measured every week using an electronic weighing scale for all the animals.

Analysis of Blood glucose level and serum lipid profiles

The rats were starved for 12 hours and then the Blood glucose level and Body weight were measured every week using a one-touch glucometer by collecting the blood from the rat tail vein. The glucometer was validated before use. On the 1^{st}, 30^{th}, 60^{th}, and 90^{th} days, blood was collected from the retro-orbital plexus of the rats under mild anaesthesia using capillary tubes containing heparin to estimate lipid levels. The serum was separated by centrifugation (5 min at 5000 rpm). On the 90^{th} day, whole blood was used to determine the HbA1c, Insulin and HOMA IR levels using the biochemistry autoanalyzer method.

Assays for enzymatic and non-enzymatic antioxidant parameters in tissue homogenates

At the end of the experiment, Part of the liver was homogenised in 0.1 M phosphate buffer (pH 7.4), and the homogenate was centrifuged at 5000 rpm for 10 min at 4 ◦a modified colourimetric protocol determined C. Then the Reduced glutathione (GSH) levels.[27] An aliquot of the liver homogenate was precipitated with 20% TCA (200 µL) and centrifuged for 5 min at 2000 rpm and 4 ◦C. After that, 50 µL of the supernatant was mixed with 100 and 50 µL of 1 M phosphate buffer and 5 mM dinitrothiocyanobenzene (DNTB). The LPO assay was carried out by measuring TBARSs as outlined by Shagirtha et al.[28] Superoxide dismutase (SOD) activity in the tissue (liver) homogenate was assessed following a protocol described by Kakkar et al.,[29] while a method outlined by Sinha [30] was used to determine catalase (CAT) activity in the liver homogenate.

Assay for α-glucosidase activity

Following their sacrifice while sedated, the rat's intestines were cleaned out. After carefully scraping the small intestine's mucous membrane lining onto an ice plate, it was mixed with phosphate buffer (pH 6.8) at a volume ratio of 5:1 (buffer: mucous membrane lining) and kept at 4ºC. The mixture was centrifuged at 5000 rpm for 20 minutes at 4 ºC. The supernatant was stored at -20ºC before being used in the α-glucosidase activity experiment. As directed by the manufacturer, the enzyme was tested using Sigma Aldrich kits (Art. No. MAK123).[31]

Histopathological analysis

At the end of the study on the 90th day rats were euthanised using a carbon-di-oxide chamber and rat Liver, kidney, pancreas, aorta, omental, and retroperitoneal adipose tissue were collected and fixed in 10% formaldehyde solution. All the tissue sections were embedded in paraffin and stained with haematoxylin and eosin (H&E). Pathophysiological changes were observed under a light microscope with a total magnification of 40x.

Statistical analysis

All the data were analysed using the SPSS software version. 26.0. Results were statistically analysed via a one-way analysis of variance (ANOVA) and a posthoc test Bonferroni's was performed. All experimental data are expressed as the mean of at least three replicates ± Standard Deviation (SD). Significant differences were accepted at * $p < 0.05$, * * $p < 0.001$, $^{\#}$ p value <0.05 compared to the metformin group and $^{\$}$ p value < 0.05 compared to the acarbose group.

PHASE - III

Molecular Docking

Molecular docking facilitates drug development by identifying the structural interactions between ligands and receptor proteins. These receptor proteins were chosen because of their crucial functions in diabetes mellitus, Hypolipidemic activity and in maintaining glucose homeostasis. This study involves the docking of ligands of *H. macroloba* against α-amylase, α-Glucosidase, Human GLUT1 in complex with inhibitors and HMG-CoA reductase as inhibitors. The docking analysis was performed using Auto Dock vina 4.0 .

To evaluate the binding affinity of ligands of *Halimeda macroloba* and standard drugs against the proposed protein targets.

Gas chromatography-mass spectrometry (GC-MS) analysis

HME (Hydroalcoholic extract of *H.macroloba*) was subjected to gas chromatography-mass spectrometry (GC-MS) examination using a GC-MS (Modal; Agilent technologies 7890A/ MS5975C) fitted with a variable flow(VF) – 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter, and 0.25 mm film thickness. An electron ionization device with an ionization energy of 70eV was employed for GC-MS detection. As a carrier gas, helium gas (99.99%) was utilized at a steady flow rate of 1 millilitre per minute. The temperature of the mass transfer line and the

injection line was set at 240°C and 200°C, respectively. The oven was set to hold at 80°C for two minutes at a rate of 10°C per minute, and then at 240°C for six minutes. Two millilitres of the samples' water solution were manually added to the split-less mode, with a mass scan of 50–600 amu and a split ratio of 1:40. Peak area normalization was used to indicate the relative fraction of each extract ingredient. Using the National Institute of Standards and Technology (NIST) library's database, which has over 62,000 spectral patterns, the mass spectrum of the plant extracts was interpreted. The compounds' spectra were contrasted with those found in the library database of the National Institute of Standards and Technology (NIST).[32]

Retrieval of 3D Structures of Receptor Proteins

The three-dimensional (3D) receptor protein structures of α-amylase - (PDB:1OSE), α-Glucosidase - (PDB:5K5N), HMG-CoA reductase - (PDB: 1DQ9) and Human GLUT1 in complex with inhibitor - (PDB: 5EQG) were obtained in pdb format from the RCSB Protein Data Bank (https://www.rcsb.org/) and xyz coordinates of 14.146x-4.022x-33.188 for PDB ID: 1OSE. 40x40x40 and xyz coordinates of 22.422x37.044x12.138 for PDB ID: 5K5N. The grid box size is 68x66x63 and xyz coordinates of 11.261x-3.011x-31.188 for PDB ID: 1DQ9. The grid box size is 58x51x68 and xyz coordinates of 10.142x-4.012x-29.32 for PDB ID: 5EQG. Which gives sufficient space for the ligand rotation and translation. The results obtained from Auto Dock were analysed to study the binding energy and the interaction of the docked structure

Refinement of Receptor Proteins

Before docking investigations, Auto Dock vina 4.0 software was used to improve the receptor proteins' protein structures. Ligands and water molecules were eliminated to develop and improve the receptor proteins. Additionally, the energy of the receptor proteins was decreased and 3D protonation was carried out with the force field gradient value set at 0.05.

Ligand Selection and Database Preparation

The compounds isolated from the GCMS analysis were selected to be analysed against the four proteins. The chemical structure of the highest peak compounds was obtained from the PubChem repository (https://pubchem.ncbi.nlm.nih.gov). Ligand optimization was performed using Merck Molecular Force Field, and MGL (Molecular Graphics Laboratory) Tools 1.5.7 was used to generate ligand parameters.

Molecular Docking

Molecular docking analysis was used to determine the most effective inhibitors of α-amylase, α-Glucosidase, Human GLUT1 in complex with inhibitors and HMG-CoA reductase. We used Auto Dock Vina 4.0 for the analysis. This process was completed by analysing Binding Interactions, Predicting Binding Modes and Optimizing Lead Compounds

By comparing POAP (Probability Occupancy Analysis Protocol), five docking runs were carried out to analyse each docking sequence to ensure high performance. Twenty high-peak compounds were screened with four proteins: α-amylase, α-glucosidase, HMG-CoA reductase, and Human GLUT1 in complex with an inhibitor.

Molecular Dynamic Simulation (MDS)

MD simulations were executed for 500ns duration using Desmond 2022 version in the Schrodinger Maestro Platform in Ubuntu OS for the target protein α-Glucosidase and ligand with the top hits from docking. An octahedral waterbox with an edge distance of 10 Å was briefly specified to fit the protein. Forty-seven potassium (K^+) and 40 chloride (Cl^-) ions were placed using the Monte Carlo method to neutralise the system. Energy minimization was set to perform a maximum of 50,000 steps with the steepest descent algorithm. Before proceeding, the system's maximum force (Fmax) needed to be below 1,000 kJ mol^{-1} nm^{-2}. The equilibrated system was then subjected to the 500-ns production MD runs (two replicates for each system).

All the trajectories were used for the calculation and we used the Desmond platform visualizing option to analyse the trajectories. For root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) calculations, we used reference structures drawn from average structures from the trajectories spanning 200 to 500 ns and fitted to the protein backbone. To determine clusters of conformation, we used the RMSD cut-off of 0.15 nm. Clustering was done for the last 400 ns, and every 500th frame was analysed. We computed the solvent-accessible surface area (SASA) in Desmond and for plotting RMSD, the radius of gyration (Rg), and SASA over time, we used the simple moving average of 100 data points.[33]

6. RESULTS

Evaluation of Acute Toxicity Study of Seaweed *H.macroloba*

S.No.	Parameters	Inference	
		Sample A	Sample B
1	Alkaloids	+++	+++
2	Steroids	---	---
3	Terpenoids	---	---
4	Tannins	+	+
5	Saponins	+++	+
6	Flavonoids	++	++
7	Phenols	+++	+++
8	Coumarins	++	+
9	Quinones	---	---
10	Glycosides	+++	+++

Sample A: Maceration Extract; Sample B: Soxhlet Extract

Sample A: Maceration Extract; Sample B: Soxhlet Extract

+++ **:**Phytocomponents present at higher concentrations.

++ **:**Phytocomponents present at medium concentration.

+ **:**Phytocomponents present at lower concentrations.

Table 1 Preliminary Qualitative phytochemical test of hydroalcoholic extract from *H.macroloba*

Table 2 Quantitative phytochemical test of hydroalcoholic extract from *H.macroloba*

S. No.	Parameters	Inference	
		Sample A (Maceration)	Sample B (Soxhlet)
1	Phenols at 750 nm	68.3	67.59
2	Alkaloids at 470 nm	148.61	147.34
3	Tannins at 715 nm	57.02	56.21
4	Saponins at 544 nm	38.27	34.31
5	Flavonoids at 510 nm	122.54	118.83

Sample A: Maceration Extract; Sample B: Soxhlet Extract

Acute toxicity study

Throughout the first four hours of continuous observation and the first twenty-four hours of the acute toxicity investigation, no deaths happened. Moreover, the animals were monitored twice daily throughout the study (14-day) period without interruption, there was no fatal consequence after 14 days. The morphological characteristics of the nose, eyes, fur, and skin seemed normal. There were no unusual behaviours seen, such as drooling, diarrhoea, or fatigue. No difference in body weight between the experimental group and the control group, which was regarded as normal. Additionally, breathing and the consumption of food and water were normal. No sedation or drowsiness was noted after the administration of the HME extract. Rats in the study didn't demonstrate any adverse consequences. So, the conclusion that can be drawn from this is that HME was safe at 50, 300, and 2000 mg/kg, b.wt. It was determined that the LD50 result exceeded the 2000 mg/kg limit test dose because there was no mortality data for all doses. No toxic effects were shown in the normal group of rats which was treated with Distilled water.

Haematological Parameters:

Table 3 included a tabulation of the impact of HME on the Wistar albino rats' haematological parameters. Red blood cells (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), neutrophils, and band cells all showed a modest rise. This has shown significant (<0.05) changes was contrasted with the group under normal management. The remaining haematological markers, including haemoglobin, Lymphocytes, Eosinophil, Basophil, and Monocytes were insignificant (>0.05) when compared with normal control.

Table 3 Effects of HME on haematological parameters of female Wistar Albino rats treated for 14 days

Parameter	Group I (Normal control) N=3	Group II (50mg/kg) N=3	Group III (300 mg/kg) N=3	Group IV (2000mg/kg) N=3
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
TC	5133.33 ± 57.73	6366.67 ± 152.75**	8100 ± 100**	6333.33 ± 152.75**
RBC	7.81 ± 0.01	6.94 ± 0.03**	7.81 ± 0.03	7.31 ± 0.02**
Hb	14.63 ± 0.15	14.73 ± 0.25	15.10 ± 0.61	14.10 ± 0.44
PCV	38.33 ± 1.15	39.50 ± 0.17*	41.17 ± 0.25**	38.40 ± 0.60
MCV	49.80 ± 0.10	56.03 ± 0.81**	53.83 ± 1.36*	53.83 ± 2.25*
MCH	18.73 ± 0.15	20.40 ± 0.50*	19.93 ± 0.15	19.37 ± 0.72
MCHC	37.50 ± 0.10	36.70 ± 0.26*	36.9 ± 0.20	36.90 ± 0.30
Platelet	7.87 ± 0.01	5.94 ± 0.40**	8.38 ± 0.37	7.59 ± 0.60
Neutrophil	6.0 ± 0.10	1.87 ± 0.15	3.47 ± 0.25	7.33 ± 3.41
Lymphocytes	92.87 ± 0.15	95.33 ± 0.45	91.97 ± 1.76	92.33 ± 1.96
Eosinophil	0.80 ±0.10	1.53 ± 0.25	2.23 ± 0.31	1.47 ± 1.11
Basophil	0.03 ± 0.05	0.10 ±0.10	0.03 ± 0.06	0.07 ± 0.06
Monocytes	7.80 ± 0.10	1.23 ± 0.66	2.0 ± 1.05	0.47 ± 0.35
Band cells	12.33 ± 0.15	13.10 ± 0.20	15.17 ± 0.42**	13.90 ± 1.11

Values are expressed as mean ± S.D. (n = 12). Statistical analysis was done by one-way ANOVA followed by Bonferroni's t-test. **P < 0.01 compared to control group, *P < 0.05 compared to control group

Biochemical Analysis

The Biochemical parameters are depicted in Table 4. The parameters include Urea blood, Creatinine, Albumin, Bilirubin, SGOT (Serum glutamic oxaloacetic transaminase) and SGPT (Serum glutamate pyruvate transaminase) were insignificant (>0.05) when weighed against the typical control group, and there were tiny variations in Total protein, globulin, AG ratio and ALP which has shown significant (<0.05) changes when compared with the normal group but the values were within the normal ranges.

Table 4 Effects of HME on biochemical parameters of female Wistar Albino rats treated for 14 days

Parameter	Group I (Normal control) N=3	Group II (50mg/kg) N=3	Group III (300mg/kg)N=3	Group IV (2000mg/kg) N=3
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Urea blood	33.33 ± 1.50	32.67 ± 1.53	31.0 ± 2.0	32.67 ± 2.08
Creatinine	0.53 ± 0.06	0.39 ± 0.08	0.50 ± 0.10	0.46 ± 0.13
Total protein	7.80 ± 0.10	7.13 ± 0.15**	0.23 ± 0.06	7.67 ± 0.25
Albumin	4.17 ± 0.06	4.17 ± 0.06	3.8 ± 0.36	4.13 ± 0.21
Globulin	3.47 ± 0.15	2.90 ± 0.10*	0.13 ± 0.06*	3.43 ± 0.25
AG Ratio	1.02 ± 0.01	1.05 ± 0.01*	0.06 ± 0.01**	1.03 ± 0.01
Bilirubin total	0.23 ± 0.06	0.27 ± 0.06	0.23 ± 0.06	0.23 ± 0.06
SGOT	125 ± 2	125.33 ± 3.21	125.67 ± 3.06	124.33 ± 3.06
SGPT	39 ±1	38.33 ± 1.53	35.67 ± 1.53	38.33 ± 1.53
Alkaline Phosphate	121 ± 1.0	97.0 ± 1.0**	92.33 ± 3.06**	100.67 ± 1.53**

Values are expressed as mean ± S.D. (n = 12). Statistical analysis was done by one-way ANOVA followed by Bonferroni's t-test. **P < 0.01 compared to control group, *P < 0.05 compared to control group

Gross morphology:

The outcome of gross morphology demonstrated that no lesions or anomalies were discovered on any of the female Wistar Albino rats' vital organs.

Histopathological Analysis:

Histopathological analysis of the Liver, Kidney and Pancreas of Wistar albino rats was Performed on the 14th day. The findings are shown in Figs. 1, 2, and 3. In all dosages of the extract-treated group, the liver's multiple slices revealed normal hepatocytes together with normal portal triads, sinusoidal spaces, and central venous system. The liver parenchyma of the rats in the normal control group was similarly normal. The renal biopsy sections derived from the extract-treated groups exhibited nearly normal glomerulus size and shape, tubular cells, and Bowman's space. Additionally, a comparison with the normal control group revealed similar results. While the control group's pancreatic sections displayed normal architecture, the group that received extract treatment additionally displayed normal architecture in their pancreatic acini and islets of Langerhans, along with normal histology.

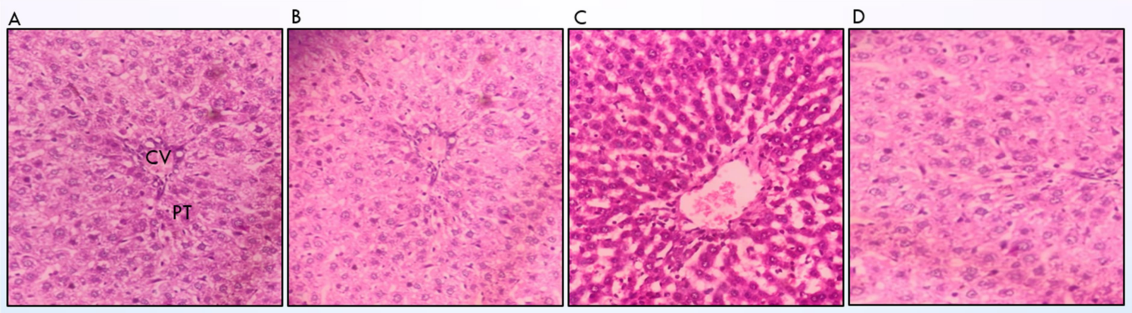


Fig-1: Light photomicrographs of hematoxylin and eosin (H&E) sections of the liver. (40 × magnification) A) Normal control, B) HME (*Halimeda macroloba* extract) 50 mg/kg BW, C) HME 300 mg/kg BW, D) HME 2000 mg/kg BW. PT: portal triad, CV: central vein

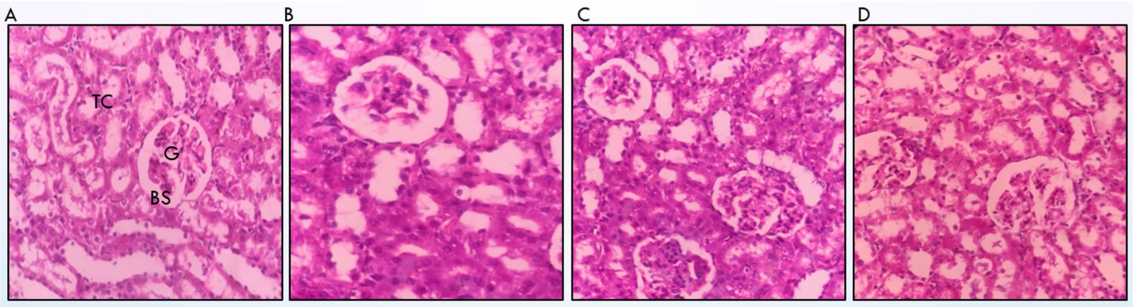


Fig-2: Light photomicrographs of hematoxylin and eosin (H&E) sections of the Kidney. (40 × magnification) A) Normal control, B) HME 50 mg/kg BW C) HME 300 mg/kg BW, D) HME 2000 mg/kg BW. G: glomeruli, BS: bowman space, TCs: tubular cells,

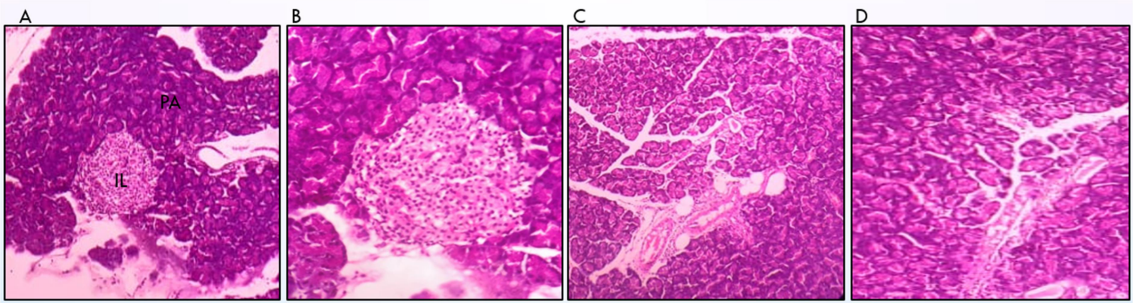


Fig-3: Light photomicrographs of hematoxylin and eosin (H&E) sections of the Kidney. (40x magnification) A) Normal control, B) HME 50 mg/kg BW, C) HME 300 mg/kg BW, D) HME 2000 mg/kg BW. ILs: islets of Langerhans, PA: pancreatic acini

Biochemical changes are correlated with histopathological alterations in the pancreas, kidneys, and liver. The liver, kidney, and pancreas showed no histopathological alterations, despite slight changes in haematological and biochemical markers.

Evaluation of Chronic Toxicity Study of Seaweed *H.macroloba*

The biochemical and haematological parameters of all the group animals on both the 90th and 180th days have not shown any abnormalities in all the doses. All the group rats have shown non-significant when compared to normal control groups.

Histopathological Analysis:

A histopathological study was carried out to confirm the biochemical and haematological changes to observe any cellular-level structural changes. The heart, liver, kidney, pancreas, and spleen in all animals, ovaries from female animals and testis from male animals were treated with HME, control group, satellite control and the satellite high dose (1000 mg/kg of HME) was observed and showed normal architecture similar to the control group. So, the NOAEL was found to be 1000 mg/kg BW of HME.

Evaluation of Antidiabetic, hypolipidemic and antioxidant activity of seaweed *Halimeda macroloba* in Wistar albino rats

Table 5 displays the impact of the HME on the fasting blood glucose levels (FBG) of rats with diabetes caused by STZ. In contrast to the normal glycaemic rat controls, we showed that experimental rats' FBG and BW significantly increased and decreased when administered 45 mg/kg BW of STZ and 110 mg/kg Nicotinamide. Furthermore, compared to diabetic rats that were left untreated, FBG significantly decreased after receiving treatments with the HME and conventional medications. Furthermore, rats that received the extract showed a considerable improvement in BW when compared to diabetic rats that were not treated. This shows that HME has better glycaemic control.

Table 5: Effect of the HME (Hydroalcoholic extract of *H. macroloba*) on Fasting Blood Glucose levels and body weight (BW) changes (in Grams) in rats with STZ-NA-induced diabetes.

	Normal Control	Diabetic Control	HME 100 mg	HME 200 mg	Metformin	Acarbose	Atorvastatin + metformin
Day 0	98.33±6.861	95.33 ±2.422	95.67±3.266	93.83±4.355	90.33±2.160	94.00±5.621	95.17±7.782
Day 3	98.33±7.421	329.83±71.848	320.17±60.234	369.83±80.517	352.00±70.163	347.17±64.648	363.83±74.160
Day 7	97.67±7.174	332.67±72.896	297.17±53.011	347.83±80.817	336.17±71.317	328.50±60.089	342.33±74.484
Day 14	97.00±6.986	336.50±73.913	279.83±51.759	326.33±77.871	322.50±69.209	310.50±57.249	325.00±74.897
Day 21	96.33±7.501	339.83±74.327	267.00±54.490	304.67±75.841	291.00±63.122	293.83±58.297	308.00±79.236
Day 28	96.17±7.731	342.00±76.811	253.00±52.907	282.33±68.269	293.17±65.786	276.50±60.998	290.17±79.215
Day 35	96.00±7.563	344.83±78.111	240.33±51.263	265.67±68.986	261.00±57.002	258.83±58.714	275.50±78.584
Day 42	96.33±7.992	347.33±79.867	226.00±46.277	249.50±63.677	260.50±63.789	243.17±57.610	258.00±73.569
Day 49	96.50 ±8.216	350.00±80.085	216.67±45.085	235.67±63.761	247.00±59.029	224.17±59.267	247.17±74.405
Day 56	97.17±7.111	351.50±79.515	200.67±39.682	218.17±63.521	233.50±57.667	208.83±53.708	232.00±72.878
Day 63	97.83±6.555	354.00±79.388	185.50±38.135	200.17±61.121	219.50±57.075	189.17±50.487	216.00±71.395
Day 70	97.17±6.940	356.00±78.509	174.17±34.822	187.17±60.204	202.17±55.217	173.00±48.658	199.67±69.793
Day 77	97.33±6.743	356.17±76.311	159.67±29.330	171.33±56.020	189.50±49.995	158.83±44.170	183.00±64.894
Day 84	97.50±7.176	357.17±75.632	144.00±23.715	162.17±54.271	173.83±45.963	144.50±41.230	167.67±0.649
Day 90	98.00±6.841	358.83±74.540	134.67±22.097	152.50±49.314	159.67±44.451	135.67±38.124	156.83±58.215

Results expressed as mean±SD (n=6). Statistical analysis was done in One way ANOVA and post hoc Bonferroni analysis was done. * value <0.05 as compared to diabetic control, ** p value < 0.001 as compared to the diabetic control

Table 6: Effect Of HME (Hydroalcoholic extract of *H. macroloba*) On Serum Metabolic Indexes In rats with STZ-NA induced Diabetes on Day 90

Groups	HbA1C %	Insulin (mU/L)	HOMA IR
Normal Control	4.91±0.62	13.25±0.20	57.75±4.64
Diabetic Control	6.66±0.22	5.04±0.01	100.96±27.98
HME 100 mg	4.72± 0.18** $^{\#\$}$	12.93±0.55** $^{\#\$}$	91.14±31.96*
HME 200 mg	4.03±0.15**	13.87±0.05**	74.16±7.11**
Metformin	4.01±0.17	14.25±0.43	83.46±16.54
Acarbose	4.13±0.25	13.93±0.09	84.01±23.73
Atorvastatin+ metformin	4.06±0.18	14.08±0.25	98.47±37.60

Results are expressed as mean ± SD (n=6). Statistical analysis was done in One way ANOVA and post hoc Bonferroni analysis was done. ** p value < 0.001 as compared to the diabetic control, $^{\#}$ p value <0.05 as compared to metformin group, $^{\$}$ p value < 0.05 as compared to acarbose group

STZ-NA administration to rats decreased the insulin levels and increased the HbA1c and HOMA IR levels. HME-treated rats exhibited an Increase in insulin levels and a decrease in Hba1c and HOMA IR levels showing higher significance than standard control rats which was depicted in Table 6

Effect of HME on Lipid Profiles in Rats with STZ-NA-induced diabetes

When comparing untreated diabetic rats to normal control rats, the data showed a substantial drop in HDL cholesterol (Table 7) and a significant increase in LDL cholesterol (Table 7), total cholesterol (Table 7), and serum triglycerides (TGs) (Table 7). When compared to untreated diabetic control, the HME (at 100 and 200 mg/kg BW), metformin alone, atorvastatin combined with metformin and acarbose significantly ($p<0.001$) reduced TC, TGs, and LDL cholesterol while increasing HDL cholesterol, when comparing to the Atorvastatin and metformin combination group HME 200 mg group shows higher significance ($p<0.05$) in HDL cholesterol levels and TC HME 100 mg group shows higher significance than the atorvastatin and metformin combination group, demonstrating the hypolipidemic activity of HME.

Table 7: Effect of Oral administration of HME (Hydroalcoholic extract of *H. macroloba*) on Lipid Profiles (HDL, LDL, TC and TG) in STZ –NA induced diabetes

	HDL (mg/dl)				LDL (mg/dl)				TC (mg/dl)				TG (mg/dl)			
Groups	**Day 0**	**Day 30**	**Day 60**	**Day 90**	**Day 0**	**Day 30**	**Day 60**	**Day 90**	**Day 0**	**Day 30**	**Day 60**	**Day 90**	**Day 0**	**Day 30**	**Day 60**	**Day 90**
Normal Control	47.19±0.83	47.34±0.81	47.50±0.84	47.68±0.86	47.30±0.76	47.37±0.77	47.65±0.92	47.95±1.22	116.56±2.85	117.54±2.41	116.83±2.16	115.88±1.74	78.35±4.08	86.23±21.40	91.15±33.09	95.93±44.31
Diabetic Control	47.68±0.63	34.75±1.83	28.88±0.95	20.42±0.88	47.84±0.67	68.18±2.42	81.97±1.83	99.01±1.14	116.04±2.95	154.83±4.03	198.74±1.69	256.68±3.16	77.25±5.45	128.73±6.19	157.54±3.03	186.21±2.30
HME 100 mg	47.89±0.64	46.74±0.95**	47.13±1.02**	47.44±1.00**	47.72±0.85	47.78±0.87**	47.98±0.91**	48.16±0.97**	116.60±3.77	137.68±1.62** #	133.84±1.98** #	131.07±1.33** #	79.92±5.99	108.82±2.79*	97.13±2.67**	88.30±2.97**
HME 200 mg	47.22±0.83	47.28±1.49**	48.11±1.66**#	48.52±1.76**#	47.86±1.14	47.00±0.79**	46.50±0.59**	45.93±0.68**	114.81±2.88	125.35±0.98**	121.82±1.20**	118.88±1.95**	78.41±5.35	105.98±3.41*	94.06±1.68**	83.28±1.95**
Metformin	47.55±0.65	45.11±1.15	45.50±0.99	45.84±0.93	47.43±0.69	47.03±0.73	46.77±0.79	46.33±0.61	114.46±3.11	127.65±1.49	124.83±1.42	121.99±0.92	77.41±5.89	108.20±3.66	97.42±2.35	87.25±3.23
Acarbose	48.17±0.71	44.92±0.62	45.26±0.61	45.48±0.72	47.19±0.83	46.84±0.85	46.25±0.70	45.63±0.53	113.89±2.21	125.31±1.54	122.20±1.65	120.25±2.12	79.17±5.41	105.71±2.81	95.41±2.11	86.62±3.56
Atorvastatin+ metformin	47.43±0.69	45.10±0.66	45.33±0.63	45.55±0.62	47.77±1.04	47.43±1.13**	47.14±1.05**	46.71±1.04**	112.61±1.24	124.75±1.31**	123.50±0.67**	121.74±1.05**	79.35±7.26	106.01±2.80*	95.63±2.66**	84.20±2.95**

Results are expressed as mean ± SD (n=6). ** p value < 0.001 as compared to the diabetic control, # p value <0.05 as compared to the atorvastatin + metformin group

Effect of antioxidant levels in Rats with STZ-NA-induced diabetes

We discovered that the HME demonstrated dose-dependent antioxidant activity by markedly raising the levels of the non-enzymatic antioxidant GSH and the activities of the liver enzymatic antioxidants, such as CAT and SOD. When compared to the antioxidant activities of the diabetic control group, both doses of HME have antioxidant activities and it was higher at HME 200

mg/kg BW. In contrast, rats treated with the HME exhibited significantly ($p<0.001$) reduced amounts of MDA, the stable by-product of lipid peroxidation than diabetic rats who were not treated. In summary, these findings showed that the HME demonstrated notable antioxidant effects in vivo in the liver of rats with diabetes induced by STZ-NA, indicating that the HME could aid the liver if employed as a hypoglycemic drug to treat diabetes which was depicted in Table 8.

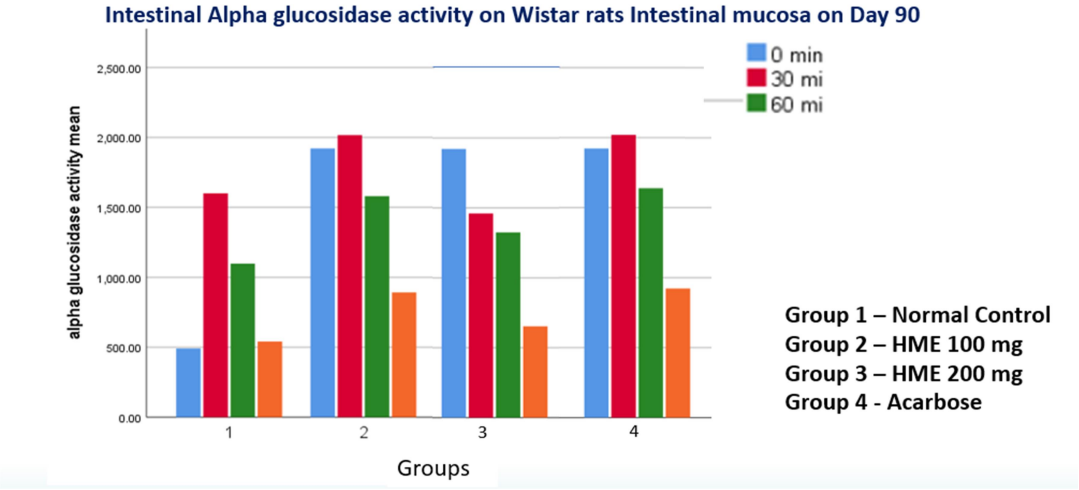
Table 8: Oxidative stress biomarkers in the liver of diabetic rats treated with the HME on Day 90

Groups	SOD (U/ml)	Catalase (U/ml)	Reduced Glutathione (GSH) (µg/ml)	TBARS (µg/ml)
Normal Control	0.28±0.003	0.22±0.023	0.11±0.002	0.08±0.001
Diabetic Control	0.23±0.015	0.18±0.010	0.93±0.039	0.16±0.009
HME 100 mg	0.31±0.003**	0.22±0.005**	0.13±0.003**	0.06±0.003**
HME 200 mg	0.32±0.001**	0.27±0.005**	0.16±0.002**	0.07±0.005**
Metformin	0.28±0.001	0.21±0.002	0.11±0.001	0.08±0.001
Acarbose	0.28±0.003	0.21±0.003	0.11±0.003	0.08±0.004
Atorvastatin+ metformin	0.28±0.003	0.21±0.002	0.11±0.002	0.08±0.002

Results are expressed as mean ± SD (n=6). ** p value < 0.001 as compared to the diabetic control

Effect of Intestinal α – Glucosidase activity in rats treated with HME on STZ-NA-induced Diabetes

The intestinal alpha-glucosidase activity was tested in the rat's intestinal mucosa at 4 time points. This has clearly shown that HME 100 have similar effects as acarbose and HME 200 mg is higher than the acarbose at all time points. This shows that HME has better alpha-glucosidase activity in all time points than acarbose



** p value < 0.001 as compared to the acarbose group

Effect of HME on Liver Histology

A normal hepatic architecture with portal triad, central vein, and hepatocytes was seen in photomicrographs of liver tissue sections taken from normal control rats. Followed by the administration of STZ-NA intraperitoneally the untreated diabetic control rats experienced significant severe periportal inflammation (PI), severe degeneration, and congested vessels with severe intrahepatic inflammation (CI) and steatosis. As expected, the 100 mg HME group rats showed

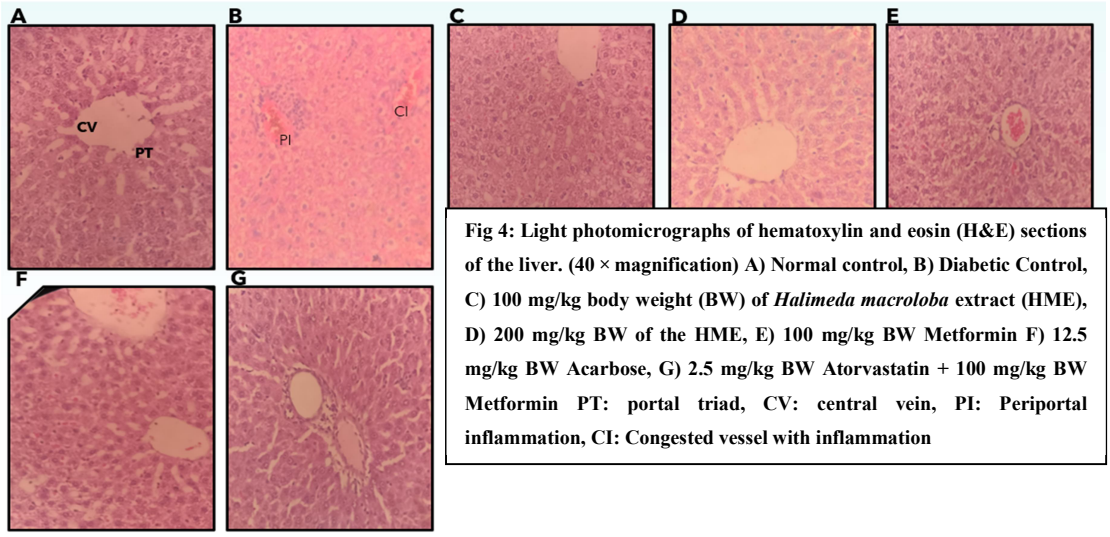


Fig 4: Light photomicrographs of hematoxylin and eosin (H&E) sections of the liver. (40 × magnification) A) Normal control, B) Diabetic Control, C) 100 mg/kg body weight (BW) of *Halimeda macroloba* extract (HME), D) 200 mg/kg BW of the HME, E) 100 mg/kg BW Metformin F) 12.5 mg/kg BW Acarbose, G) 2.5 mg/kg BW Atorvastatin + 100 mg/kg BW Metformin PT: portal triad, CV: central vein, PI: Periportal inflammation, CI: Congested vessel with inflammation

healed liver sinusoids with normal hepatocytes and mild congestion, while the 200 mg HME group showed normal liver sinusoids and hepatocytes with less steatosis. Other groups display normal hepatocytes, while the Metformin Group displays slight hepatic congestion and inflammation which is shown in Fig 4.

Effect of HME on Kidney Histology:

A typical renal architecture with glomeruli, bowman space, renal tubules, and tubular cells was visible in a photomicrograph of kidney tissue sections taken from normal control rats. after administering STZ-NA, the untreated diabetic rats showed nodular glomerular sclerosis, significant intrarenal bleeding, and severe intrarenal inflammation. When compared to the diabetic control rats that were not treated, it is noteworthy that oral administration of the HME 100 mg/kg BW resulted in mild renal globular and tubular regeneration with mild thickened globular basement membrane, and 200 mg/kg BW group rats also showed normal glomeruli with lobular regeneration and typical tubular epithelial vascularization. The standard group experienced similar effects; however, glomerular basement thickening was observed in the metformin group as depicted in Fig 5.

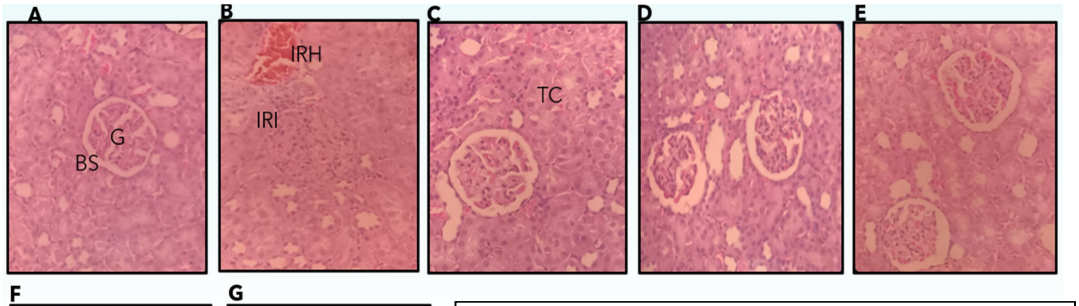


Fig 5: Light photomicrographs of hematoxylin and eosin (H&E) sections of the Kidney. (40 × magnification) A) Normal control, B) Diabetic Control, C) 100 mg/kg body weight (BW) of *Halimeda macroloba* extract (HME), D) 200 mg/kg BW of the HME, E) 100 mg/kg BW Metformin F) 12.5 mg/kg BW Acarbose, G) 2.5 mg/kg BW Atorvastatin + 100 mg/kg BW Metformin G: Glomeruli, BS: Bowman space, RTs: Renal Tubules, TC: Tubular cells, IRH: Intra Renal Haemorrhaging, IRI: Intra renal inflammation

Effect of HME on Pancreatic Tissue Histology

A photomicrograph of pancreatic tissue sections of normal control rats revealed normal well-perfused pancreatic tissues with active islets of Langerhans, pancreatic acini and well-outlined acini cells (Fig. 6). STZ- NA administration results in chronic acinar inflammation with atrophy of islet cells in the diabetic control rats. Whereas both HME-treated groups show normal pancreatic tissues, islets of cells and pancreatic acini with regenerative signs were visualized in the entire parenchyma. Another standard group also shows normal pancreatic acini and the islet of Langerhans.

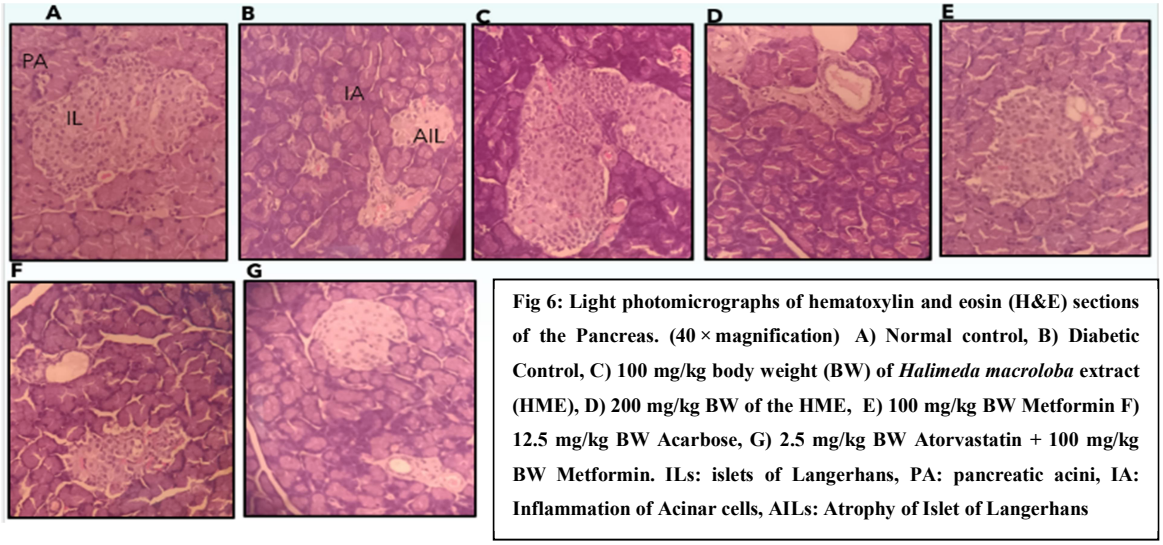


Fig 6: Light photomicrographs of hematoxylin and eosin (H&E) sections of the Pancreas. (40 × magnification) A) Normal control, B) Diabetic Control, C) 100 mg/kg body weight (BW) of *Halimeda macroloba* extract (HME), D) 200 mg/kg BW of the HME, E) 100 mg/kg BW Metformin F) 12.5 mg/kg BW Acarbose, G) 2.5 mg/kg BW Atorvastatin + 100 mg/kg BW Metformin. ILs: islets of Langerhans, PA: pancreatic acini, IA: Inflammation of Acinar cells, AILs: Atrophy of Islet of Langerhans

To evaluate the binding affinity of ligands of *Halimeda macroloba* and standard drugs against the proposed protein targets.

GCMS

The GC–MS chromatograms of *H.macroloba* compounds are presented in Fig 7. Analysis reveals that the compound with the highest area percentage (16.48%) is 3 Ergosta-4,6,22-trien-3.beta.-ol. On the other hand, the compound with the lowest area percentage (0.20%) is Methyltris (trimethylsiloxy) silane. Following these most of the identified compounds constitute the prominent phytochemicals derived from this seaweed, as shown in Table 20 **(refer to Figure 7**).

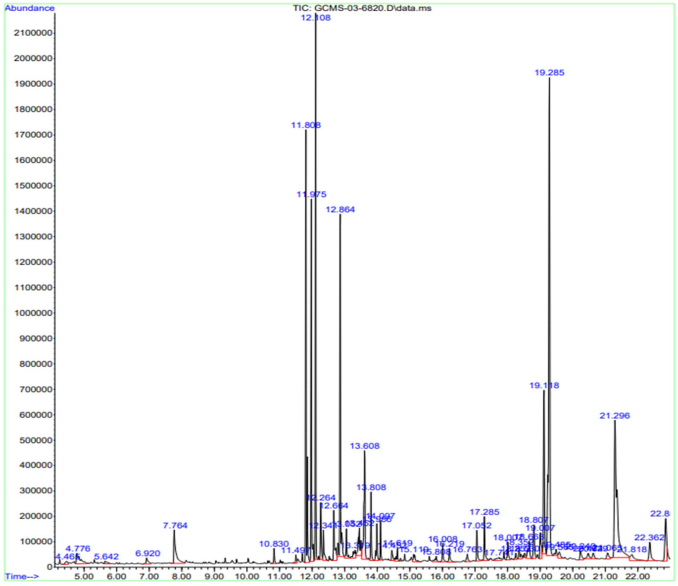


Figure 7: GC-MS chromatogram of hydroalcoholic extract of the seaweed *Halimeda macroloba*

Molecular Docking:

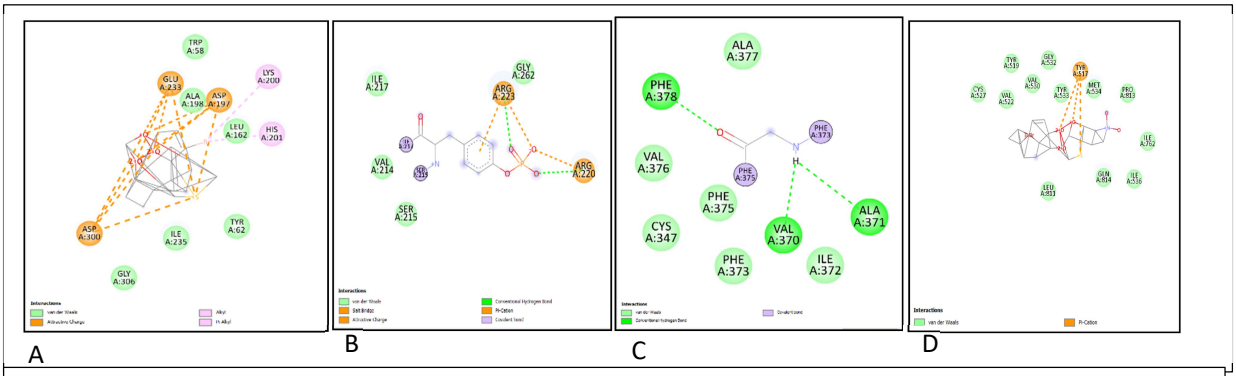
Auto Dock Tools was used to assist with the docking validation and docking assessments. The screening's objective is to evaluate the target, which contains a lot of compounds. The screening was performed in 20 ligands, and one compound—benzenesulfonic acid, 4-nitro-, 4-bromomethyl-2-adamantyl) ester (PubChem: 590094)—showed the highest binding affinity and best score against four proteins: alpha-amylase, alpha-glucosidase, human GLUT1 in complex with inhibitor and HMG-CoA reductase. Table 9 displays the outcomes of the best-performing compounds as well as the quantity of binding energy generated during the contact.

Table 9: Molecular interaction studies of H.macroloba against the target proteins

S.No	Protein Names	Compound Pubchem CID	Docking Score (kcal/mol)	Interaction Amino acids
1	α-amylase complex (PDB: 1OSE)	590094	-10.9	Glu233, Ala198, Leu162, His20, Asp300, ASP197
2	**α** - Glucosidase (PDB:5K5N)	590094	-12.4	Phe378, ARG223, ARG220, Gly262, Ile217, SER A 215
3	Human Glut 1 Complex with inhibitor (PDB:5EQG)	590094	-11.3	Phe378, Val370, Ala371, Phe A:375, Phe A:373
4	HMG CO-A Reductase (PDB: IDQA)	590094	-9.4	Tyr517, Met534,

2D Interaction of α-amylase complex (PDB: 1OSE)

The docking interactions of the ligand Benzenesulfonic acid, 4-nitro-, (4-bromomethyl-2-adamantyl) ester (Pubchem: 590094) against the protein α-amylase complex showed the binding affinity of -12.4 kcal/mol was in the interaction of Glu233, Asp A197, Ala198, Lys200, Leu162, His201, Asp300 and also the van der Waals Gly306, Ile235, Tyr62, Trp58 residues of 1OSE which was shown in 2D and 3D representation in Figure 8.



A- Figure 8: 2D interaction of ligand of *H.macroloba* with α-amylase, **B** - Figure 9: 2D interaction of the ligand of *H.macroloba* with α-Glucosidase, **C** Figure 10: 2D interaction of the ligand of *H.macroloba*with Human Glut 1 Complex with inhibitor, **D** - Figure 11: 2D interaction of the ligand of *H.macroloba* with HMG CO-A Reductase

The docking interactions of the ligand Benzenesulfonic acid, 4-nitro-, (4-bromomethyl-2-adamantyl) ester (Pubchem: 590094) against the protein α-Glucosidase complex showed the binding affinity of -10.9 kcal/mol was in the interaction with the presence of Phe378, ARG223, ARG220, Gly262, Ile217, SER A 215 and the van der Waals Gly A: 262, Val A:214, Ser215, Ile217 are the residues of 5K5N which was shown in 2D and 3D representation in Figure 9.

2D Interaction Human Glut 1 Complex with inhibitor – (PDB:5EQG)

The docking interactions of the ligand Benzenesulfonic acid, 4-nitro-, (4-bromomethyl-2-adamantyl) ester (Pubchem: 590094) against the protein Alpha-amylase complex 5EOG showed the binding affinity of -10.9 kcal/mol was in the interaction with Phe378, Val370, Ala37, Phe A:375, Phe A:373 which is an Alkyl group and the presence of Van der Waals ILE372, PHE A:373, VAL376, ALA A:377, CYS A:347, PHE A:373 residues of 5K5N.which was shown in 2D and 3D representation in Figure 10.

2D Interaction of HMG CO-A Reductase – (PDB: IDQ9)

The docking interactions of the ligand of Benzenesulfonic acid, 4-nitro-, (4-bromomethyl-2-adamantyl) ester (Pubchem: 590094) against the protein HMG CO-A Reductase complex showed the binding affinity of -9.4 kcal/mol was in the interaction with Tyr517and the presence of Van der Waals Met534, Pro813, Ile762, Ile536, Cys527, Tyr519, Gly532, Pro813, Ile762, Gln814, Leu811, Val522, Cys527 residues of IDQ9 which was shown in 2D and 3D representation in Figure 11.

Molecular Dynamic Simulation

To examine the kinetics and stability of the target protein α-Glucosidase (PDB:5K5N) and the ligand **Benzenesulfonic acid, 4-nitro-, (4-bromomethyl-2-adamantyl) ester** which has shown top hits in the docking was investigated by 500 ns of molecular dynamics simulations. Based on the observations of the RMSD, RMSF, Protein-Ligand Contacts and Ligand Trajectory results the protein and ligand demonstrated significant stability; hence it was confirmed that the test ligand is a significant inhibitor of the target protein. Based on the in-silico MDS analysis, it is proposed that the test ligand **Benzenesulfonic acid, 4-nitro-, (4-bromomethyl-2-adamantyl) ester** is a promising inhibitor molecule for **α-Glucosidase protein**.

Trajectory Analysis

Trajectory movement of the ligand over the surface of the protein during the MDS was analysed within the Desmond platform visualizing option. It was observed that, throughout the entire 500ns duration of the MDS, the test ligand did not migrate away from the binding site and maintained a stable interaction at the site of docking.

Individual interpretations components of Trajectory analysis in MDS

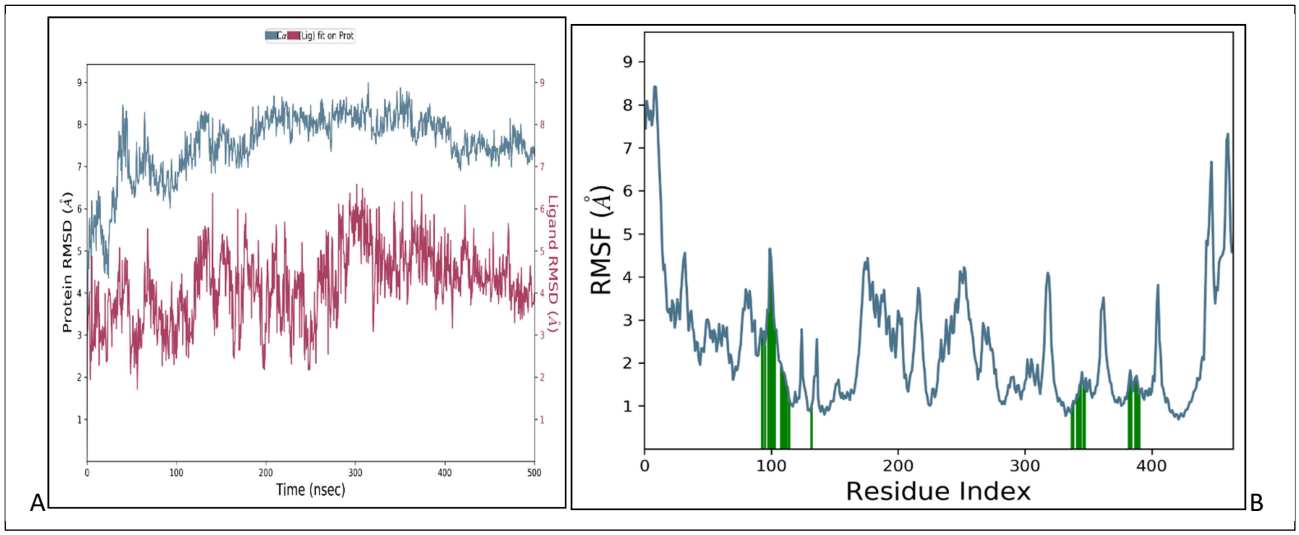
RMSD

Root Mean Square Deviations are an indication of the displacement of the atoms of the proteins and ligands during the MDS, in reference to the initial position of the atoms. A stable protein-ligand complex should ideally demonstrate a stable RMSD without huge fluctuations or changes in the

RMSD. The results of the RMSD analysis are graphically represented in **Figure 12**. It was observed that RMSD values of both the protein and ligand independently are stable throughout the 500ns duration. The protein macromolecule demonstrated an average RMSD of 8Å that fluctuated between 7Å ~ 9Å. This suggests that the protein macromolecule demonstrated an average fluctuation of 2Å after attaining stability at 50ns.

RMSF

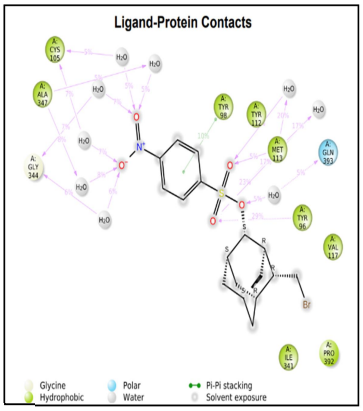
Root Mean Square Fluctuation is an indication of the fluctuation or flexibility of individual amino acid residues of the macromolecule. The results of the RMSF analysis are graphically represented in **Figure 13.** The image also highlights the residues that were in contact with the ligand directly. It was observed that the amino acid residues, that were directly in contact with the ligand demonstrated less than 2Å fluctuations, indicating that the ligand is imposing rigidity on the protein residues, causing inhibition of the activity.



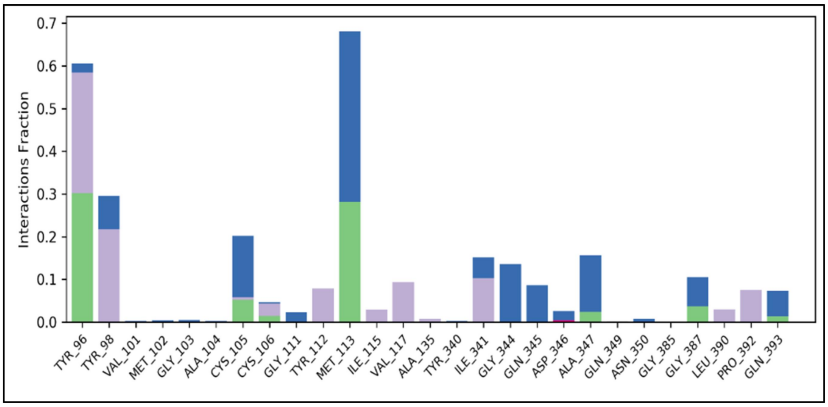
A - Figure 12: RMSD of Protein (α-Glucosidase (PDB:5K5N)) and Ligand (4-nitro-, (4-bromomethyl-2-adamantyl)) during MDS, **B** - Figure 13: RMSF of amino acid residues of proteins

Protein-Ligand Contacts

The chemical interactions between the protein and ligands were analysed and graphically represented as a structural plot **Figure 14** and as a graphical plot **Figure 15**. The formation of chemical interactions and duration of the chemical bonds between the protein and ligand play a significant role in the protein-ligand complex stability and the inhibition potential of the ligand. It was observed that, among all the residues that were in contact with the ligands, the residues, Tyr-96 and Met-113 are the residues that demonstrated the maximum duration of contact or interaction with the macromolecule. Among this, Met-113 demonstrated a total of 65% duration (for 325ns) of interaction with the sulphite group of the ligand, within which, 37% duration was Water Bridge interaction, and 28% duration was Hydrogen Bond interaction. While, Tyr-96 demonstrated a total of 29% duration (for 145ns) of interaction with the sulphite group of the ligand, which is of Hydrogen Bond interaction followed by Hydrophobic Interaction that lasted for an equal duration of 30% duration (for 150ns). These observations suggest that, although the ligand demonstrated significant polar and non-polar interactions, the most significant interactions were observed to be centred on the Sulphite group of the ligand that is forming a stable interaction with the protein and is contributing majorly towards the inhibition of the target protein.



A



B

A - Figure 14: 2D Representation of Protein-Ligand Chemical Interactions, B - Figure 15: Graphical Representation of the Chemical Interactions between Protein-Ligand

This strongly suggests that the ligand (**Benzenesulfonic acid, 4-nitro-, (4-bromomethyl-2-adamantyl) ester**) is a strong inhibitor of the target protein (**α-Glucosidase**). and this can be considered a significant inhibitor of the target protein.

7. DISCUSSION

The α-glucosidase inhibitory property of *H.macroloba* has been demonstrated by in vitro scientific endeavours.[9] To our knowledge the *H.macroloba*'s *in vivo properties* were not studied. As a result, the current study will be the first to describe the potentially harmful effects and pharmacological properties of this scientifically undiscovered marine seaweed. So, we conducted this investigation to assess the antidiabetic, hypolipidemic and antioxidant activities of HME in Wistar rats with STZ-NA-induced diabetes.

The preliminary phytochemical screening (qualitative and quantitative) of HME was conducted and the result revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, coumarins and glycosides. Thus, the preliminary phytochemical test helps in determining the class of chemical compounds present in the extracts which may lead to their quantitative and qualitative estimation and also identifying the source of pharmacologically active phytoconstituents. The concentration of pharmacologically active compounds was higher with the maceration method than with the Soxhlet extraction method.

In our study, a single administration of HME extract with increasing doses did not produce any mortality or any serious abnormities at all doses in the acute toxicity study. However, the limit test dose for the study was 2000 mg/kg, BW which did not show any severe toxic effects and mortality, thus 1/10th, 1/20th of the limit test dose was chosen as the dose (200 mg/kg, BW and 100 mg/kg, BW) for conducting an Evaluation of Antidiabetic, Hypolipidemic and Antioxidant effects of seaweed *Halimeda macroloba* study. Single-oral dose of HME in rats produced no behavioural changes and body weight changes in female rats when compared with the control group and was considered normal. Gross morphology on vital organs of female Wistar Albino rats such as the liver, kidney, pancreas, heart, lungs, stomach and ovaries showed that no lesions or abnormalities were found.

In histopathological studies, no major alterations in vital organs (liver, kidney, pancreas) were found. With these results, the hydroalcoholic extract of *H. macroloba* did not cause harm to the liver, kidney and Pancreas. Thus, it can be concluded that HME oral administration did not produce any major clinical signs and did not affect the rats throughout the study period of 14 days.

In our chronic toxicity study (OECD 452), the administration of HME extract with increasing doses did not produce any mortality or any serious abnormalities at all doses. However, the highest test dose for the study was 1000 mg/kg, BW Which did not show any severe toxic

effects or mortality. The oral ingestion of HME in rats produced no behavioural changes in both male and female rats. Gross morphology was studied to determine the presence of any visible lesion on vital organs such as the liver, kidney, pancreas, heart, lungs, stomach, spleen, testis and ovary of rats respectively and the result showed no lesions or abnormalities

In our chronic toxicity study, the doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg did not show any treatment-related abnormalities in terms of haematological, biochemical parameters and histopathological analysis. When compared to the control group no significance was observed, indicating that the administered drug at doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg is safe. The LD50 of HME was found to be more than 2000 mg/kg by acute toxicity study and No Observed Adverse Effect Level (NOAEL) was found to be 1000 mg/kg of HME.

In the HME's *in vivo* antidiabetic, hypolipidemic, and antioxidant potential of *H. macroloba* in Wistar rats with STZ—NA-induced diabetes study. The increased fasting blood glucose concentration coupled with the loss of BW recorded in STZ-NA intoxicated rats could have resulted from oxidative stress induced by STZ-NA, which agrees with findings from previous studies. The decreased BW might have resulted from increased catabolism of fats and proteins, consequently leading to a reduction in muscle mass and weight loss. Importantly, our results demonstrated a blood glucose normalization effect and improved BW in diabetic rats treated with both doses of HME.[34, 35]

Fasting insulin levels on day 90 show a significant increase in insulin levels in the HME-treated groups when compared to diabetic control. And our findings were in accordance with the ***kosal et al., 2015***[36] there was a significant decrease in insulin levels in rats after being treated with streptozotocin, our diabetic control rats also showed a decrease in insulin levels.

HbA1c on Day 90 was significantly lower ($p < .001$) in rats treated with both doses of HME respectively. Animals treated with HME 100 and 200 mg/kg BW had a significant ($p <0.05$, $p < .001$) drop in HOMA-IR on day 90. The effect of HME 100 mg/kg BW on HOMA-IR, was less significant ($p < 0.05$) when compared to HME 200 mg/kg BW. This shows the prevention of STZ-NA-induced oxidative stress and protects the β-cells resulting in significant elevation in BW, insulin secretion and a significant decrease in Fasting Blood glucose level, HOMA IR, and HbA1c. This suggests the antidiabetic effect of HME.

It is interesting to note that there was a significant difference in α-glucosidase activity in the HME 200 mg group compared to the Acarbose group. This implies that HME has a higher efficacy than Acarbose. Thus, HME could be developed into a new type of hypoglycemic agent with similar

α-glucosidase inhibitory activity. This will have the advantage of overcoming the gastrointestinal discomfort usually associated with the use of Acarbose

Several metabolic disorders including hyperlipidemia were reported to result from insulin resistance.[37] Reduced action of insulin leading to increased lipase activity with consequential release of free fatty acids from adipose tissues is the main cause of hyperlipidemia. The current study demonstrated the efficacy of the HME in modulating hyperlipidemia induced by STZ - NA administration. The untreated diabetic control rats had elevated TGs, TC, LDL-Cholesterol and decreased HDL-cholesterol levels. When compared to the untreated rats we found that both the doses of HME effectively reduced levels of TGs, TC, and LDL-cholesterol, while concurrently elevating the HDL-cholesterol levels from Day 30 and the results were consistent till the end of the study (day 90). This suggests that HME has Hypolipidemic activity. In the current study, the increased lipid peroxidation and reduction of the antioxidant enzyme activities in rats with STZ-NA induced diabetes indicated the increased generation of free radicals in the liver of untreated diabetic rats, leading to oxidative damage to membranes, proteins, and lipids with consequential decreases in endogenous levels of antioxidants (SOD, CAT, and GSH).

Interestingly, our data revealed that both doses of HME decreased hepatic MDA levels and increased hepatic SOD, CAT and GSH levels in STZ-NA-dosed rats. Overall, these results indicated that the HME exhibited significant antioxidant activities *in vivo* in the liver of rats with STZ-NA-induced diabetes, thus suggesting that the HME could have a beneficial effect on the liver if used as a hypoglycemic agent to treat diabetes. Treatment with HME and Acarbose brought about a significant reduction in the activity of intestinal α-glucosidase.

Our histological study of the livers of STZ-NA-induced diabetic untreated rats showed severe degeneration, peri portal inflammation (PI), congested vessels with severe intrahepatic inflammation (CI) and steatosis. Interestingly, the degenerative changes and severe PI, CI and steatosis were ameliorated upon treatment with both doses of HME. When compared to the standard group liver, HME group liver shows better-healed liver sinusoids. Together these results provide important insights into the protective role of the HME against liver injury. Thus, suggesting the hepatoprotective effect of the HME. Similarly, histological impairments of the diabetic control rats' kidneys showed Intrarenal haemorrhage (IRH), Intrarenal inflammation and nodular glomerular sclerosis. Those were reversed in the HME-treated group rats and showed normal renal parenchyma with lobular regeneration and typical tubular epithelial vascularization. Furthermore, the diabetic control rats' pancreas revealed chronic acinar inflammation (AI), and atrophy of islets

of Langerhans (AILs). These effects were attenuated in the pancreas by extract treatment and showed normal pancreatic acini and islet cells with regenerative signs visualized in the entire pancreatic parenchyma when compared with the diabetic non-treated rats. Conclusively, our serum and tissue biochemical analysis correlated with the histological observations and strongly suggested the therapeutic role of HME in managing DM and its associated complications. To summarize, our study provides preclinical evidence that *H.macroloba* has antidiabetic, hypolipidemic and antioxidant activity that may help to develop alternate therapy regimens for T2DM.

For drug discovery, in silico screening of natural chemicals has become essential due to expensive to execute, difficult, and time-consuming screening methods. A significant proportion of resources are lost as a result of disoriented laboratory techniques that lack structural knowledge of medications and target molecules. However, computational biology lowers a drug's chance of failing in its final stages.[38] *H.macroloba* was chosen as an antidiabetic and hypolipidemic agent source for the current investigation. Dry lab methods like molecular docking are strongly advised for a better evaluation of the binding patterns between small compounds and their associated protein binding domains (PBD) for a comprehensive grasp of the basic biochemical processes. This method could enable us to investigate the degree of ligand-receptor interaction at the atomic level. According to reports, seaweed may be useful in cancer therapy and it is used against the PLpro of SARS-CoV-2.[39,40] In the current investigation, four target proteins (α-amylase, α-Glucosidase, Human GLUT1 in complex with inhibitors and HMG-CoA reductase) were chosen because of their critical functions in regulating the body's glucose levels and cholesterol levels. The anti-diabetic and hypolipidemic properties of the compounds that were isolated from the seaweed are detailed in our in-silico investigation. To find a possible therapeutic candidate, we combined some of the in-silico techniques, including molecular docking and molecular dynamics simulation. In molecular docking, our results indicate that the lead compound, benzene sulfonic acid, 4-nitro-, 4-bromomethyl-2-adamantyl ester, had the greatest binding affinity and interacted with all target proteins efficiently.

For molecular dynamics simulations of antidiabetic efficacy, we selected the top-hit compound from the docking complex, which demonstrated a high level of binding stability and a high binding affinity and then the target protein (α-Glucosidase) and the ligand (benzene sulfonic acid, 4-nitro-, 4-bromomethyl-2-adamantyl ester) has been evaluated for its stability. It was discovered that the RMSD values of both the protein and ligand individually remained consistent during the 500 ns period. The protein macromolecule has an average RMSD of 8Å, fluctuating between 7Å and 9Å. This indicates that the protein macromolecule fluctuated by an average of 2Å

after reaching stability at 50 ns. In RMSF, it was discovered that the amino acid residues that were directly in contact with the ligand displayed less than 2Å changes. This suggests that the ligand is imposing rigidity on the protein residues, which inhibits their activity. At the docking site, the test ligand maintained a stable connection and did not move away from the binding site which was essential for inhibiting the action of α-glucosidase. This suggests that *H.macroloba* is a good lead candidate.

8. SUMMARY AND CONCLUSION

In conclusion, *H. macroloba* has significant antidiabetic, hypolipidemic, and antioxidant action based on the *in vivo* study and the *insilico* approach. It shows no toxicity in both acute and chronic toxicity studies. Its antidiabetic, hypolipidemic and antioxidant activity was demonstrated by the significant reduction of fasting blood glucose, HbA1c, Insulin Resistance, improved BW and insulin levels, a significant improvement in lipid profiles (a decrease in LDL, TG, and TC along with the increase in HDL levels) and significantly increased the antioxidant enzymes such as GSH, SOD and CAT and reduced the MDA levels against STZ-NA induced diabetic rats. Additionally, the findings of dynamic simulation and molecular docking demonstrated a higher binding affinity against the hypolipidemic and antidiabetic targets. To investigate its possible therapeutic uses for humans, more research is required on *H. macroloba*, which is the first study of its kind to include substantial unique bioactive components.

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LIST OF RESEARCH PUBLICATIONS

Related to Ph.D. work:

1. R. K, Ananthy V, Salwe KJ, Manimekalai K, Komarasamy B, Deva R. An Overview of Indian Marine Seaweeds and their Medicinal Applications. Journal of Pharmacology and Pharmacotherapeutics. 2024;15(3):221-236. doi:10.1177/0976500X241264899

Review Article

An Overview of Indian Marine Seaweeds and their Medicinal Applications

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Kavya R.[1], Vimala Ananthy[1], Kartik Janardan Salwe[1,2], K. Manimekalai[1], Boopathi Komarasamy[3,4] and Reka Deva[3,4]

Abstract

Background: Seaweeds are macroalgae containing bioactive compounds that have positively impacted the biomedical field due to polysaccharides, sterols and glycolipids, showing a diverse range of pharmacological and biological properties. Eating a diet high in marine items will lessen the risk of developing diabetes, cancer and obesity.
Purpose: The exploration of medically useful seaweeds is required to identify drugs and drug targets and reduce the risk of developing diseases.
Methodology: The PubMed search was done with the MESH terms such as seaweed and Marine algae pharmacological activity results were obtained as 748 results. We filtered out the articles and entered key terms related to our area of interest to find the results. The seaweed species in India are listed with Tamil Nadu contributing the most and Orissa the least. The type of algae is also mentioned.
Results: Marine algae have been employed in industry and medicine as a novel food with possible nutritional benefits. Furthermore, it has been demonstrated that marine algae are a rich source of naturally occurring bioactive substances with activities such as anti-diabetic, hepatoprotective, antiviral, antifungal, antibacterial, antimicrobial, antioxidant, anti-inflammatory, anti-hypercholesterolemia and hypolipidemia.
Conclusion: The current review focuses on the bioactivities and potential pharmaceutical activity of marine algae discovered in India. The present review article showed that there are many seaweeds of medical importance, and it is necessary to explore them to minimize disease progression and prolong the individual's survival.

Keywords

Brown seaweed, green seaweed, Indian seaweed, marine macroalgae, pharmacological activity, red seaweed

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Introduction

Seaweeds are types of macroalgae that can be found adhering to the seafloor in coastal waters that are rather shallow. Up to a depth of 180 m, solid substrates including rocks, dead coral, pebbles, shells and other plant materials may support the growth of organisms. In addition, estuaries and backwaters are where we may find them flourishing. They are classified as Chlorophyceae-green algae (*Ulva clathrata, Monostroma nitidum, Caulerpa* spp.), Phaeophyta-brown algae (*Saccharina japonica, Undaria pinnatifida* and *Sargassum fusiforme*) and Rhodophyceae-red algae (*Porphyra/Pyropia* spp., *Eucheuma* spp., *Kappaphycus alverazii* and *Glacilaria* spp.), and together they make up one of the most essential types of living resources. They are usually seen to stretch to depths of 30–40 m and can be found in large numbers on hard soils. India has a seaweed production capacity of 77,000 t (wet weight), and there have been reports of about 1,153 species. The red seaweeds contribute 27.0%, the brown seaweeds contribute 0.2% and the other seaweeds comprise 72.8%. There have been around 206 different

[1]Department of Pharmacology, Mahatma Gandhi Medical College & Research Institute, Sri Balaji Vidyapeeth, Pillaiyarkuppam, Puducherry, India
[2]Current affiliation: Department of Pharmacology, Sunderlal Patwa Government Medical College, Mandsaur, Madhya Pradesh, India
[3]Department of Chemistry, Pondicherry University, Kalapet, Puducherry, India
[4]Department of Pharmacology, JIPMER, Puducherry, India
Corresponding author:
Reka Deva, Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry 605006, India.
E-mail: meetdr.reka478@gmail.com

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2. Rangaraj K, Ananthy V, Singam A, et al. Phytochemical analysis and acute toxicity study of seaweed *Halimeda macroloba* using Wistar albino rats. *J Complement Integr Med*. 2024;21(4):511-519. Published 2024 Sep 5. doi:10.1515/jcim-2024-0077

Basic Research Article

Kavya Rangaraj*, Vimala Ananthy, Amrita Singam, Kartik J. Salwe, Kumarappan Manimekalai, Shanmuga Samy and Shravan Venkatraman

Phytochemical analysis and acute toxicity study of seaweed *Halimeda macroloba* using Wistar albino rats

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Abstract

Objectives: *Halimeda macroloba (H. macroloba)* a seaweed commonly known as green macroalgae is a potential source of bioactive compounds utilised in nutraceuticals and pharmaceuticals. However, there are no reliable scientific studies that describe harmful consequences, which attest to its safety. Thus, the current investigation focuses on a 14-day acute toxicity assessment of *H. macroloba* hydroalcoholic extract (HME).

Methods: HME was prepared using 70 % alcohol as solvent by the maceration method for 72 h & Soxhlet method. Phytochemical analysis was done using standard procedures, according to OECD 423 guidelines. Female Wistar albino rats fasted overnight and received a single oral dosage of 50, 300, and 2,000 mg/kg BW (Body Weight). Further rats were starved for 4 h and watched individually for every 30 minutes, then twice a day for 14 days. To determine the toxicity overall behaviour, BW, haematological, biochemical, histopathology, and gross morphology were examined.

Results: Pharmacologically active phytoconstituents were identified by phytochemical analysis. No significant abnormalities/fatalities from single-dose of HME at escalating doses. No BW or behavioural changes. The majority of the haematological and biochemical parameters were normal. Did not show any apparent changes. simultaneously, a few indicated slight variations that may or may not be caused by HME extract no lesions or anomalies in Gross morphology. Histopathological investigations revealed that HME had no adverse effects on organs.

Conclusions: HME administration at doses up to 2,000 mg/kg BW didn't result in acute toxicity/impairment to the pancreas, liver or kidney. Nevertheless, the study's limited test dose of 2,000 mg/kg, BW, didn't result in any fatalities/ adverse effects.

Keywords: acute toxicity; green macroalgae; *Halimeda macroloba*; maraine seaweed; phytochemical screening; Wistar albino rats

***Corresponding author: Kavya Rangaraj**, Research Scholar, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth, Pillalyarkuppam, Puducherry, India, E-mail: kavya07rangaraj@gmail.com
Vimala Ananthy, Kartik J. Salwe, Kumarappan Manimekalai and Shanmuga Samy, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth, Pillalyarkuppam, Puducherry, India
Amrita Singam, Vasantrao Naik Shaskiya Vaidyakla Mahavidyalay, Yavatmal, India
Shravan Venkatraman, Jipmer, Puducherry, India

Introduction

Toxicology is the study of toxins and poisons, along with their consequences and treatment. Toxicological screening is critical for the development of novel medications as well as the expansion of current compounds' therapeutic potential. According to the US Food and Drug Administration (FDA), it is necessary to evaluate novel substances in animals for pharmacological and potentially dangerous effects [1]. Seaweeds are photosynthetic algae found around the oceans. Seaweed is classified into three basic phyla: Phaeophyceae (brown algae), Rhodophyta (red algae), and Chlorophyta (green algae) [2, 3]. Traditional uses for seaweeds include food, home medicines, colours, and fertilisers. When mass food production began in the early 1900s, seaweed components were used industrially. Because of their ability to gel in foods, pharmaceuticals, and biotechnological applications, hydrocolloids like alginate, carrageenan, and agar remain the most commonly utilised components [4, 5]. Regarding the creation of substitutes for green fuel, the viability of using seaweed for biodiesel production has been investigated [6]. Seaweeds have gained popularity as nutraceuticals, or functional foods that offer dietary benefits beyond their macronutrient content, throughout the last 30 years.

PRESENTATIONS

Oral Presentation:

1. Presented oral Paper Presentation on Acute toxicity study *Halimeda macroloba"* In "National Conference on Natural Products/AYUSH System of Medicine" on 20th - 21st June 2023
2. Presented oral Paper Presentation on "GCMS and Molecular docking studies of Hydroalcoholic extract of *Halimeda macroloba as a potent anti-diabetic activity"* In "National Conference on Natural Products/AYUSH System of Medicine" conducted by PGIMER on 19th - 21st April 2024

CERTIFICATE

Awarded to

Kavya R

Presented Online Oral Presentation entitled

"Acute toxicity study of seaweed Halimeda macroloba"

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Dr. Ajay Prakash, Organizing Secretary